JRC TECHNICAL REPORTS

6th Meeting of the Advisory Board for Comparative Testing

Financial and Technical Report

Diana Charels

2012

Report EUR 25541 EN

Joint Research Centre

EURL European Union Reference Laboratory for GM Food & Feed

European Commission
Joint Research Centre
Institute for Health and Consumer Protection

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JRC75883

EUR 25541 EN

ISBN 978-92-79-26850-2

ISSN 1831-9424

doi: 10.2788/53251

Luxembourg: Publications Office of the European Union, 2012

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Printed in Italy

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Molecular Biology and Genomics

European Union Reference Laboratory for Genetically Modified Food and Feed

6th Meeting of the Advisory Board for Comparative Testing

Financial and Technical Report

19/10/2012

European Commission, Joint Research Centre
Institute for Health and Consumer Protection (IHCP)
Molecular Biology and Genomics Unit – European Union Reference Laboratory for Genetically Modified Food and Feed
Via Fermi 2749, 21027 Ispra (VA) – Italy

Certification by the beneficiary

Workshop: 6th Meeting of the Advisory Board for Comparative Testing

Date: 23-24/08/2012

Start: 09:00 (23/08/2012) End: 12:30 (24/08/2012)

Administrative arrangement: *N °SANCO/2011/FOOD SAFETY/066-GMOs*

Maximum financial assistance for a task force meeting: 4410.00 EUR

We certify that:

- The expenditure given in Annex II of the present document (i.e. 4183.31 EUR) was actually incurred, accurately accounted for and eligible under the provisions of Regulation (EC) No 926/2011,
- All supporting documents relating to these costs are available for inspection,
- We are not deriving any profit from the subsidy granted by the Commission.

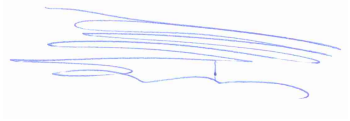
Joachim Kreysa
Head of the Molecular Biology and Genomics Unit

Date: 19/10/2012

Signature: 

Eric Cremer, Financial Officer
Unit B4 – Budget, Accounting and Competitive Activities

Date: 19/10/2012

Signature: 

CONTENT

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Scientific Officer

Scientific and technical approval:
M. Mazzara
Competence Group Leader

Compliance with EU-RL Quality System:
S. Cordeil
Unit Quality Manager

1. Introduction

In accordance with the work programme 2012[1], the European Union Reference Laboratory for Genetically Modified Food and Feed (EU-RL GMFF) has organised the 6th Meeting of the Advisory Board for Comparative Testing on 23 and 24 August 2012, in Ispra (Italy). Invitations to the meeting were sent to all members of the Advisory Board for Comparative Testing.

In February 2012 Philippe Corbisier from the Reference Materials Unit at the Institute for Reference Materials and Measurements (IRMM, Geel, BE) joined the Advisory Board for Comparative Testing. With the exception of Isabel Taverniers and Lotte Hougs all members of the Advisory Board for Comparative Testing attended the meeting. The following topics were discussed:

- The draft report of the fourth round of comparative testing ILC-EURL-GMFF-CT-02/11
- The standard deviations for proficiency testing of the fifth comparative testing round ILC-EURL-GMFF-CT-01/12 containing oilseed rape event GT73 and maize event 59122
- Preparation of test materials: decision about the test materials for 2013 and brainstorming about future test materials

The final report regarding the fourth round of comparative testing ILC-EURL-GMFF-CT-02/11 will shortly be sent to DG SANCO.

This report presents the minutes of the meeting and the financial report thereof.

2. 6th Meeting of the Advisory Board for Comparative Testing

The 6th Meeting of the Advisory Board for Comparative Testing was held in Ispra on 23 and 24 August 2012. The members of the Advisory Board for Comparative Testing who attended the meeting are listed in Annex I.

The meeting focused mainly on the draft report of the fourth comparative testing round ILC-EURL-GMFF-CT-02/11. The draft report was uploaded on the comparative testing workspace https://englnet.jrc.ec.europa.eu/ABCT on 17 August 2012 to receive feedback from the Members of the Advisory Board. The minutes of the meeting of the Advisory Board and the executive summary of the final report can be found below.

[1] Administrative arrangement: *N °SANCO/2011/FOOD SAFETY/066-GMOs*

2.1. Minutes of the sixth meeting of the members of the Advisory Board for comparative testing

Aim of the meeting: To provide assistance to the EU-RL GMFF for the organisation of their comparative testing rounds.

Present: H. Broll, B. China, P. Corbisier, D. Charels, H. Hird, S. Jacchia, K. Kolodziej, J. Kreysa, M. Maras, M. Sandberg, M. Schulze
Date: 23-24/08/2012
Abbreviations:
CT: comparative testing

2.1.1. Comparative Testing Report on the Detection and Quantification of Maize Events GA21, TC1507 and MIR604 - Comparative testing round: ILC-EURL-GMFF-CT-02/11

The main point of discussion at the meeting was the draft report of the fourth comparative testing round (see below for an executive summary of the report).

Comparative testing round: ILC-EURL-GMFF-CT-02/11

Executive Summary

The European Union Reference Laboratory for Genetically Modified Food and Feed, established by Regulation (EC) No 1829/2003(1), organised a comparative testing round for National Reference Laboratories nominated under Regulation (EC) No 882/2004(2) and Regulation (EC) No 1981/2006(3), for members of the European Network of GMO Laboratories, for Official control laboratories and for laboratories from third countries which had volunteered to participate.

In accordance with Article 32 of Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules, the European Union Reference Laboratory for Genetically Modified Food and Feed shall organise comparative testing and shall ensure an appropriate follow-up of such testing.

The design and execution of the comparative testing round was in accordance with the ISO 17043 Standard(4). The European Union Reference Laboratory for Genetically Modified Food and Feed is accredited according to the ISO 17043 Standard 'General requirements for proficiency testing'(4).

The test items used in the comparative testing round ILC-EURL-GMFF-CT-02/11 were produced in-house. Pioneer Overseas Corporation provided a coarsely ground powder of TC1507 (unique identifier DAS-Ø15Ø7-1) seeds. Syngenta Biotechnology, Inc. provided devitalised seeds of maize events MIR604

(unique identifier SYN-IR6Ø4-5) and GA21 (unique identifier MON-ØØØ21-9). Participants were required to screen two test items denoted maize powder levels 1 and 2, for the presence of maize events 3272, Bt11, Bt176, 59122, GA21, MIR604, MON 810, MON 863, NK603 and TC1507. Any events detected were then to be quantified. In September 2011, a total of 159 laboratories were invited to participate in ILC-EURL-GMFF-CT-02/11, and subsequently 102 laboratories registered for this comparative testing round. Test items were shipped to participants at the end of October 2011 in plastic containers containing approximately 5 g of flour. Ninety-three laboratories from 40 countries returned results, which fell into the following groups:

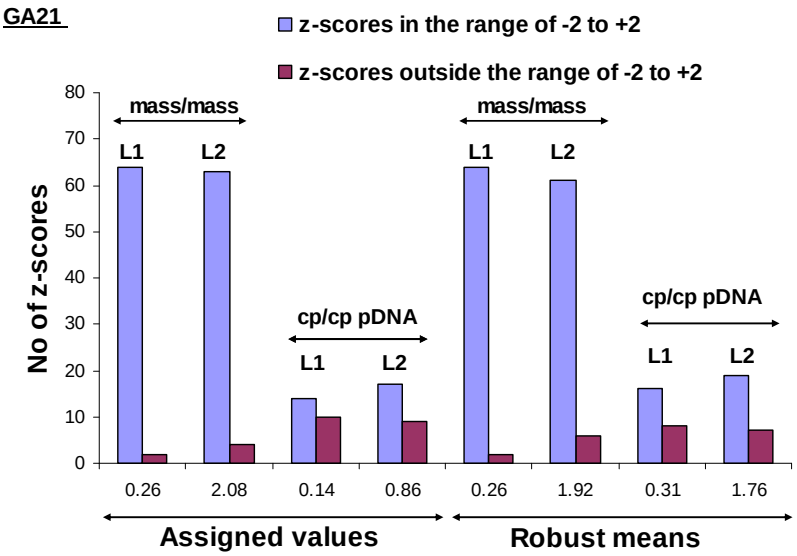
1. 3 were National Reference Laboratories nominated only under Regulation (EC) No 882/2004 (group 1),
2. 29 were National Reference Laboratories nominated only under Regulation (EC) No 1981/2006 (group 2),
3. 30 were National Reference Laboratories nominated under both Regulations (group 3),
4. 7 were only members of the European Network of GMO Laboratories (group 4),
5. 8 were only official control laboratories (group 5),
6. 16 were laboratories from third countries (group 6).

Eight laboratories, of which seven were National Reference Laboratories (groups 1 to 3) and one was an official control laboratory (group 5), submitted results in both measurement units. Two National Reference Laboratories and one European Network of GMO Laboratories (group 4) registered twice but submitted both sets of results in the same measurement unit. Nine laboratories including one National Reference Laboratory (group 3), one European Network of GMO Laboratory and seven laboratories from a third country (group 6) did not submit results. The Food Safety and Quality Unit of IRMM managed the on-line registration and submission of results.

Participants could report the results in either mass/mass % or copy/copy %. The European Union Reference Laboratory for Genetically Modified Food and Feed calculated the robust means (μ_R) of the maize powder levels 1 and 2 test items in mass/mass % and in copy/copy %. All data were log-transformed and then robust statistics were applied to obtain a robust mean [5, 6, 7]. In addition, values (μ) were assigned by the European Union Reference Laboratory for Genetically Modified Food and Feed on the basis of the data from the homogeneity study[8] (mass/mass % data) and digital Polymerase Chain Reaction[9] (copy/copy % data). The homogeneity, stability and digital Polymerase Chain Reaction studies were conducted at the European Union Reference Laboratory for Genetically Modified Food and Feed. These data were included in the uncertainty budget.

The target standard deviation for comparative testing $\hat{\sigma}$ was fixed at 0.20 ($\log_{10}$ value) for the maize events TC1507 and MIR604 and 0.25 ($\log_{10}$ value) for event GA21 by the Advisory Board for Comparative testing. These target standard deviations were used to derive z-scores for the participants' results. An overview of the robust means and number of z-scores in the range of -2 to +2 is given in Figure 1.

a)



b)

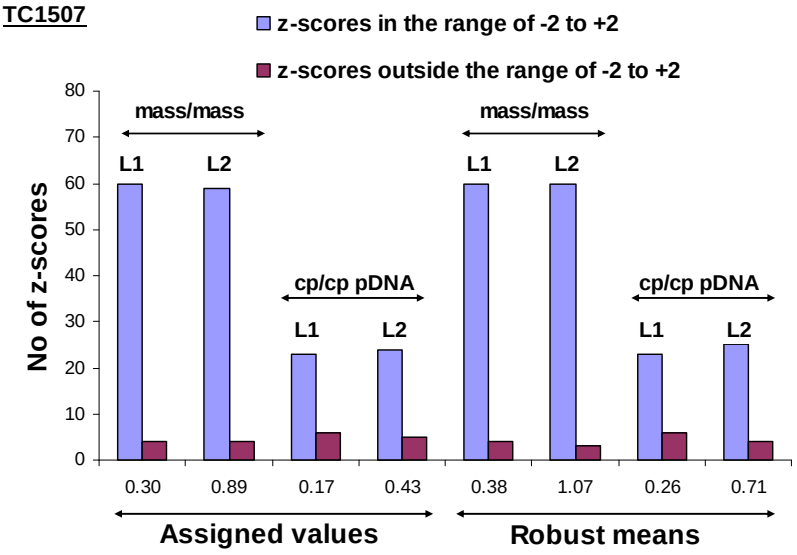


Figure 1. Overview of z-scores calculated on the basis of assigned values and robust means for maize events GA21 (a), TC1507 (b) and MIR604 (c). mass/mass = mass/mass %, cp/cp = copy/copy %, L1 = level 1, L2 = level 2.

c)

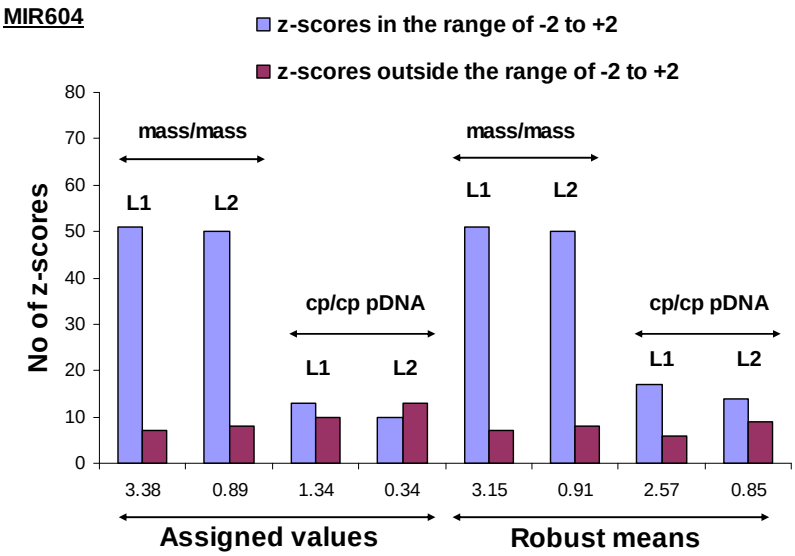


Figure 1 (continued). Overview of z-scores calculated on the basis of assigned values and robust means for maize events GA21 (a), TC1507 (b) and MIR604 (c). mass/mass = mass/mass %, cp/cp = copy/copy %, L1 = level 1, L2 = level 2.

In this fourth comparative testing round greater than 86 % of participants gained a satisfactory z-score in the range of -2 to +2 for the results expressed in mass/mass % for both maize powder levels 1 and 2 regardless of the GM event. However, a lower percentage (43 – 86 %) of z-scores within the working range of -2 to +2 was calculated for those participants that expressed the results in copy/copy %.

Participants' assessment of results in relation to measurement uncertainty needs to be improved because about 53 % of participants provided information on measurement uncertainty in a complete and consistent manner.

Conclusions

In this fourth comparative testing round greater than 86 % of participants gained a satisfactory z-score in the range of -2 to +2 for the results expressed in mass/mass % for both maize powder levels 1 and 2 regardless of the GM event. The assigned values derived from the homogeneity study conducted at the EU-RL GMFF were very close to the robust means expressed in m/m %. However, a lower percentage (43 – 86 %) of z-scores within the working range of -2 to +2 was calculated for those participants who expressed the results in copy/copy %.

In this regard there is an obvious disparity between the consensus value calculated on the basis of participants' results expressed in copy/copy % and the value assigned in-house by the EU-RL GMFF. The cause for this seems to lie in the fact that those participants did not take into account the biological factors when converting mass/mass % to copy/copy %.

There is still a need for harmonisation with respect to the reporting of the measurement uncertainty because about 53 % of participants provided information on measurement uncertainty in a complete and consistent manner.

2.1.2. Decision on standard deviation for comparative testing for EURL-GMFF-CT-01/12

The standard deviation for comparative testing $\hat{\sigma}$ was set to:

- $\hat{\sigma}$ = 0.20 for event oilseed rape GT73 also called RT73
- $\hat{\sigma}$ = 0.20 for maize event 59122

2.1.3. Preparation of test materials

2.1.3.1. Decision about the test materials for 2013

1. ILC-EURL-GMFF-CT-01/13

Two test materials of processed food (*e.g.* flour made from baked biscuits) containing wheat and maize will be used as test items. The material will be ground and spiked with maize GM events MON 863 x MON 810 (ERM®-BF417) and 98140 (ERM®-BF427) in a known concentration. IRMM will provide the GM events to the EU-RL GMFF. Participants shall:

- perform species identification: maize, soybean, oilseed rape and prepare a table listing the presence/absence of each species;
- screen for a list of maize events provided by the EU-RL GMFF (3272, Bt11, Bt176, 59122, GA21, MIR604, MON 810, MON 863, NK603, 1507, MON 88017, MON 89034, 98140, MIR162) and quantify those GM events that were detected.

2. ILC-EURL-GMFF-CT-02/13

Official control laboratories will be asked to provide real samples of rice products (noodles, biscuits,…) to the EU-RL GMFF for the preparation of test items. Alternatively, the EU-RL GMFF would purchase a commercial product as starting material. The material will be ground and spiked with soybean event 356043 (ERM®-BF425) in a known concentration. IRMM will provide the GM events to the EU-RL GMFF. Participants will be informed that a rice sample is provided and shall:

- perform species identification: maize, soybean, oilseed rape, rice and prepare a table listing the presence/absence of each species;
- identify and quantify the added soybean GM event.

As in 2012, a series of tests will be performed on each test item to determine its homogeneity and stability. The absolute quantification of the GM content will be performed using digital PCR. In 2013 next generation sequencing might also be employed, but mainly on an experimental basis.

2.1.3.2. Brainstorming about future test materials

It could be envisaged to prepare four concentrations for a given CT round and to provide different concentrations to different participants. Participants are only provided with bottle numbers. Bottle numbers are randomly labelled to avoid that a participant might be able to deduce the concentration on the basis of the bottle number. The EU-RL GMFF would then randomly select two bottles for each participant. When a certain concentration would be selected, this concentration would be excluded from selection for the second sample to be tested.

For future comparative testing rounds (i.e. after 2013) IRMM could produce an extra concentration (e.g 0.7 m/m %, 1.3 m/m %,…) of a new GM event for which they have been asked to produce Certified Reference Materials. This new GM event could subsequently be used in a CT round.

2.1.4. Actions to be taken

a. Introduce all necessary changes in the draft report on the fourth CT round and submit it to the hierarchy [D. Charels].
b. Actions regarding observed overestimation of results expressed in cp/cp % [D. Charels]:

    i. Compare robust means with values assigned in-house. Take necessary actions in case the variation between both values is too large.
    ii. Educate participants who overestimated the GM content expressed in cp/cp % - foresee a training course, provide a guidance document,…

c. Analyse the results of EURL-GMFF-CT-01/12 and draft the preliminary report [D. Charels].
d. The Food and Environment Research Agency will be asked to provide a recipe for the preparation of biscuits [D. Charels].

2.1.5. References

1. European Commission (2003). Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. *Off. J. Eur. Union* L 268: 1-23
2. European Commission (2004). Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. *Off. J. Eur. Union* L 191: 1-52
3. European Commission (2006). Regulation (EC) No 1981/2006 of 22 December 2006 on detailed rules for the implementation of Article 32 of Regulation (EC) No 1829/2003 of the European Parliament and of the Council as regards the Community reference laboratory for genetically modified organisms. *Off. J. Eur. Union* L 368: 99-109
4. ISO/IEC 17043:2010 Conformity assessment – General requirements for proficiency testing

5. Analytical Methods Committee (2004). GMO Proficiency Testing: Interpreting z-scores derived from log-transformed data. RSC. *AMC Technical Brief.* No. 18. December 2004
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7. Analytical Methods Committee (1989). Robust statistics – How not to reject outliers Part 1. Basic Concepts. *Analyst* 114: 1359-1364
8. R204GP40/EURL Record homogeneity ANOVA. EU-RL GMFF internal quality document.
9. Dube, S., Qin, J., Ramakrishnan, R. (2008). Mathematical Analysis of Copy Number Variation in a DNA Sample Using Digital PCR on a Nanofluidic Device. *PLoS ONE* 3: e2876. doi:10.1371/journal.pone.0002876

3. Financial report

Annex II presents the outcome of the calculation made for the reimbursement of the participants in the meeting of the Advisory Board for Comparative Testing according to the "implementing rules regarding workshops", as provided by DG SANCO. All participants in the 6th meeting of the Advisory Board for Comparative Testing were reimbursed according to the rules defined above. The outcome of the calculation is 4183.31 Euros out of the 4410.00 Euros originally planned in the work programme for a task force meeting. This amount represents the eligible expenditure of the EU-RL GMFF for the 6th meeting of the Advisory Board for Comparative Testing.

Annex I
Participants' list

MS	Organisation	Participant
BE	Scientific Institute of Public Health (IPH)	CHINA, Bernard
DE	Federal Institute for Risk Assessment (BfR)	BROLL, Hermann
BE	Institute for Reference Materials and Measurements (IRMM)	CORBISIER, Philippe
DE	State office for Consumer protection and Food Safety (LAVES), State Food surveillance Laboratory (Lebensmittelinstitut) Braunschweig	SCHULZE, Manuela
SE	National Food Administration	SANDBERG, Martin
UK	The Food and environment Research Agency (FERA)	HIRD, Heather Jayne

Annex II
EU-RL GMFF expenditures for the 6th Meeting of the Advisory Board for Comparative Testing

Legal basis : Commission Regulation (EU) n° 926/2011
(Official Journal L 241 of 17/9/2011)

Place
Ispra IT

Workshop Dates
23-24/08/2012

Currency	MS	Participant	Departure	Arrival	Travel			Total travel paid by EU-RL	Total Travel in EUROS	Nr Hours	Daily allowance	N	Maximum allowance	Hotel costs	Hotel costs in EUROS	TOTAL (Travel + allowance + hotel)
					Air	Train	Car									
EUR	DE	BROLL HERMANN	24-08-12 15:05	22-08-12 18:35	334.10			794.1	**334.10**	45.00	95.00	2.00	**190.00**		**270.00**	794.1
EUR	BE	BERNARD CHINA	24-08-12 14:40	22-08-12 16:45	135.48			595.48	**135.48**	46.00	95.00	2.00	**190.00**		**270.00**	595.48
EUR	DE	SCHULZE MANUEL	26-08-12 18:20	22-08-12 6:40	216.17	78.00		889.17	**294.17**	60.00	95.00	2.00	**190.00**		**405.00**	889.17
SEK	SWE	SANDBERG MARTIN	24-08-12 15:00	22-08-12 16:00	4541.00			1003.91	**543.91**	47.00	95.00	2.00	**190.00**		**270.00**	1003.91
GBP	UK	HIRD HEATHER	24-08-12 15:30	22-08-12 11:25	286.36	57.28		900.65	**440.65**	44.00	95.00	2.00	**190.00**		**270.00**	900.65
					5513.11	**135.28**	**0.00**	**4,183.31**	**1,748.31**				**950.00**		**1,485.00**	**4,183.31**

European Commission
EUR 25541 EN – Joint Research Centre – Institute for Health and Consumer Protection

Title: 6th Meeting of the Advisory Board for Comparative Testing

Author(s): Diana Charels

Luxembourg: Publications Office of the European Union

2012 – 16 pp. – 21.0 x 29.7 cm

EUR – Scientific and Technical Research series – ISSN 1831-9424

ISBN 978-92-79-26850-2

doi: 10.2788/53251

Abstract
In the frame of Regulation (EC) No 882/2004, the European Union Reference Laboratory for Genetically Modified Food and Feed (EU-RL GMFF) has the duty to organise comparative testing rounds for National Reference Laboratories (NRLs). The EU-RL GMFF is assisted in its duties by a so-called Advisory Board for Comparative Testing. The aim of the Advisory Board is to provide support to the organisation of the comparative testing rounds and to give guidance on the approach for participants' assessment and on participants' results and performance.
Within two months after the Advisory Board meeting has taken place a financial and technical report has to be submitted to DG SANCO. The following topics were discussed at the Advisory Board meeting of 23 and 24 August 2012:

- The draft report of the fourth round of comparative testing ILC-EURL-GMFF-CT-02/11
- The standard deviations for proficiency testing of the fifth comparative testing round ILC-EURL-GMFF-CT-01/12 containing oilseed rape event GT73 and maize event 59122
- Preparation of test materials: decision about the test materials for 2013 and brainstorming about future test materials

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Publications Office

ISBN 978-92-79-26850-2

9 789279 268502